The Bioscan 8(4): 1545-1549, 2013 (Supplement on Genetics & Plant Breeding)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

MULTIVARIATE ANALYSIS OF GENETIC DIVERGENCE AMONG INDIAN MUSTARD (*BRASSICA JUNCEA* L. CZERN & COSS) GENOTYPES IN RELATION TO OIL QUALITY TRAITS

BINOD KUMAR[1], ANIL PANDEY[2] AND SANJAY KR. SINGH[3]
[1&2]Department of Plant Breeding and Genetics, Rajendra Agricultural University, Pusa - 848 125, Samatipur
[3]Department of Soil Science, Rajendra Agricultural University, Pusa - 848 125, Samatipur
e-mail: binod_gpb022@rediffmail.com

KEYWORDS
Indian mustard
Genetic divergence
Clusters analysis
Oil quality

Received on :
17.09.2013

Accepted on :
06.12.2013

***Corresponding author**

ABSTRACT
The pattern of distribution of genotypes in different clusters exhibited that geographical diversity was not related to genetic diversity as genotypes of same geographical region were grouped into different cluster and vice-versa. Amongst seven, clusters II were mono-genotype clusters, whereas cluster IV was the largest having 16 genotypes followed by cluster III and V involved 12 and 5 genotypes, respectively. Maximum divergence exhibiting clusters II and VII may be utilized through inter varietal hybridization to exploit high degree of genetic diversity between them. Solitary genotypes (RAURD 172) in cluster II might have resulted from geographical barriers preventing gene flow or intensive selection for adaptive gene complexes. Noteworthy is that cluster V exhibited high cluster mean for palmitic acid, oleic acid and low erucic acid; Cluster VII show low value of cluster mean for linolenic acid, high value for linoleic acid and erucic acid; whereas, high value of cluster mean for stearic acid in cluster I and high cluster mean for linolenic acid and oil content showed in cluster II and VI respectively, reflected probability of getting better segregants and primary recombinants expected to more, in case if the genotypes of these clusters will be used in hybridization programme.

INTRODUCTION

Indian mustard *Brassica juncea* (L.) Czern & Coss. is the second largest oilseed crop in India after soybean. It is cultivated in *rabi* (post-rainy) season mainly in Northwest India and contributes nearly 27 per cent to edible oil pool of the country. Major mustard growing states are Rajasthan, M.P., U.P., Haryana, Gujarat, Bihar, Punjab, West Bengal and Assam in India. Genetic diversity plays an important role in plant breeding because hybrid between lines of diverse origin generally display a great heterosis than those between closely related strains (Singh, 1983) which permits to select the genetically divergent plants to obtain the desirable recombination of the segregating generation. Multivariate analysis is important tool in quantifying the degree of divergence between biological population at genotypic level and to assess the relative contribution of different components to the total divergence both at intra- and inter-cluster levels (Jatasra and Parada, 1978; Zahan *et al.* 2008). Therefore, the present research work was to identify divergent parents for hybridization program, which would provide superior segregates in mustard genotypes. The material from diverse geographical origin of the crop species can help to ensure conservation of co-adapted gene complexes (Frankel *et al.*, 1995). The application of genetic variation can also be manipulated either for selecting superior genotypes or to be utilized as parents for the development of future cultivars through hybridization.

MATERIALS AND METHODS

The experiment was conducted with forty six genotypes of Indian mustard were grown in Randomized Block Design with three replications at the research farm of Tirhut College of Agriculture, Dholi, Muzaffarpur (Rajendra Agricultural University-Pusa) Bihar during *rabi* season of 2010-11. Each genotype was sown in a plot consisting of three rows of 5m length in three replications with inter and intra row spacing of 30cm x 10cm. Presecribed recommended package of practices for Indian mustard were followed to raise a healthy crop. The experimental data were recorded on five randomly selected competitive plants of each genotype in all the replications. The seeds of different lines/genotypes were drawn for estimation of oil content by prescribed standard procedure. The fatty acid procedure were identified and characterized as, Palmitic acid, Stearic acid, Oleic acid, Linoleic acid, Linolenic acid and Erucic acid and their mean values were subjected to various statistical and biometrical analyses. Fatty acid methyl esters were prepared from the seeds of each line, following the procedure described by AOAC (1990). The GLC was used for the analysis of fatty acid using a NUCON-9657, GLC instrument equipped with column as follows, 2m, 1/8" 0.d, 2mm i.d., mesh 100-200, SP 2300+2310 with flame ionization detector (FID). Nitrogen gas used as a carrier gas and the temperature of injection port and detector were maintained at 230^{0}C and 240^{0}C and the temperature programming during the analysis, it was held initially at 120^{0}C for 1 minute, and then to 230^{0}C @ of 10^{0}C / min. The peak fatty acids were identified based on their retention time using standard fatty acid esters. The percentage oil content of rapeseed mustard seeds was determined using a Foss-tecator

Near Infrared Reflectance Spectroscopy (FT-NIRS). Over 4g seeds of each intact sample were scaned in a 36nm inner diameter ring cup. The oil content calibration equation was determined using a modified partial least square regression method (Wu *et. al*. 2006). The genetic divergence was estimated by Mahalanobis (1936) D^2 statistics and the grouping of the genotypes into clusters were done using Tocher's method (c. f. Rao, 1952).

RESULTS AND DISCUSSION

In any crop improvement venture, genetically distant parents are needed for crossing programme. This is to create the required genetic diversity between genotypes in terms of gene frequencies which may result for heterotic group and/or transgreesive segregants. The analysis of variance was highly significant among the divergent genotypes for all the seven traits under study, which revealed the presence of considerable variability among the studied genotypes (Table 1). This suggested that adequate scope is available for selection and breeding. Mean of quality traits of the genotypes are presented in Table 2. Fatty acid content were separated by GLC as palmitic, stearic, oleic, linoleic, linolenic, erucic acid and oil content of the genotypes ranged from 1.815-3.412, 0.120-1.070, 9.008-14.364, 14.399-20.142, 6.490-12.918, 36.191-54.849 and oil content was 35.370-39.410 respectively. The highest content of oleic acid was obtained from the genotypes RAURD 246 and RAURD 35 whereas, the lowest was by genotype RAURD 172, for linoleic acid the highest value genotypes was RAURD 168 and RAURD 170 and the lowest value for linoleic acid in RAURD 190, for linolenic acid the highest value was recorded by the genotype RAURD 172 and JD-6 whereas the lowest was recorded by the genotype RAURD 171. Similarly the highest value for erucic acid was exhibited in Varuna and RAURD 170 and the lowest value for erucic acid was recorded in RAURD 221, which could be used as parental material for improvement of erucic acid content of the seed oil of Indian mustard. Generally, these results indicate that those traits which had wide range of variations will serves for breeding and selection of the trait desired. Erucic acid constitutes the major proportion of the total fatty acids which is in agreement with the findings of Teklewold (2005) and Genet *et.al.* (2004). In this study the range of oil content of the genotypes is almost similar with the oil content of the released varieties (Alemayehu, 1990). Forty six genotypes, the basis of Mahalanobis D^2 following Euclidean method for clustering, were grouped into seven clusters with cluster-wise variable number of genotypes (Table 3) developed by various centres and located at different geographical locations suggesting considerable amount of genetic diversity in the material. Amongst seven, clusters II were mono-genotype clusters, whereas cluster IV was the largest having 16 genotypes involving varieties/ strains from various centers. Similarly, cluster III involved 12 genotypes, cluster V and VI having 5 and cluster I and VII involved only 2 genotypes, respectively. The pattern of distribution of genotypes in different clusters exhibited that geographical diversity was not related to genetic diversity as genotypes of same geographical region were grouped into different cluster and vice-versa. The intra and inter -cluster distances (Table 4) and the mean performance of the clusters (Table 5) were used to select genetically diverse genotypes. The highest genetic

Table 1: Analysis of variance for seven quality characters of 46 Indian mustard genotypes

Characters	Mean sum of squares		
	Replications	Treatments	Error
Degree of freedom	02	45	90
Palmitic Acid	0.013	0.363**	0.003
Stearic Acid	0.009	0.146**	0.001
Oliec Acid	0.019	4.754**	0.003
Linoleic Acid	0.036	3.471**	0.007
Linolenic Acid	0.005	3.675**	0.002
Erucic Acid	0.413	38.169**	0.672
Oil Content (%)	0.011	2.244**	0.014

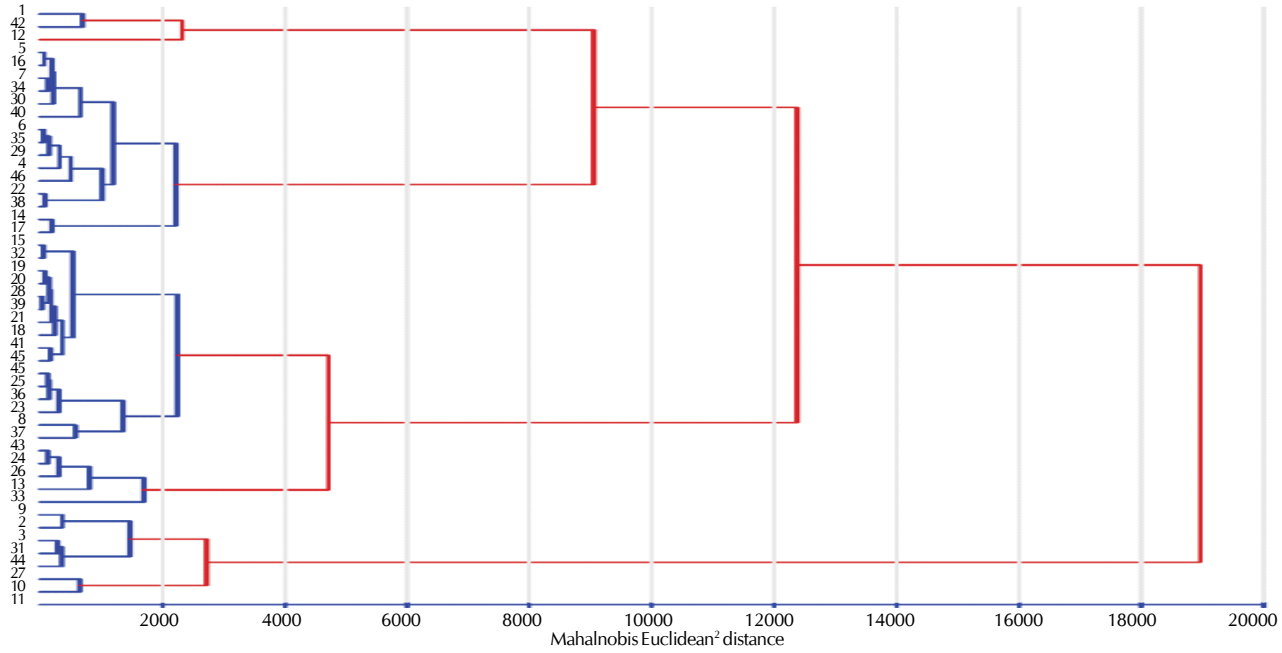

Figure 1: K Wards minimum variance dendrogram which shows the distribution of the 46 Indian mustard genotypes

Table 2: Mean performance for seven quality characters of 46 Indian mustard genotypes

S. No.	Character	Fatty acid profile						
		Palmitic acid	Stearic acid	Oliec acid	Linoleic acid	Linolenic acid	Erucic acid	Oil content (%)
1.	RAURD-34	2.118	0.800	9.611	16.079	11.208	43.188	37.583
2.	RAURD-35	1.917	0.831	14.244	17.369	8.408	46.788	38.180
3.	RAURD-89	2.661	0.918	13.141	17.745	8.661	45.329	38.983
4.	RAURD-153	2.061	0.536	10.242	16.322	9.724	40.912	36.390
5.	RAURD-154	2.342	0.729	11.933	17.286	9.381	44.159	36.703
6.	RAURD-156	2.253	0.636	11.194	16.271	9.600	46.943	36.810
7.	RAURD-164	2.254	0.912	12.176	15.818	9.436	44.732	37.313
8.	RAURD-166	2.391	0.834	12.066	16.190	11.135	47.280	38.253
9.	RAURD-168	3.040	0.917	14.217	20.142	9.495	36.797	35.857
10.	RAURD-170	2.873	0.120	13.131	19.008	7.211	51.204	38.263
11.	RAURD-171	2.646	0.338	12.123	17.140	6.490	49.570	37.687
12.	RAURD-172	1.823	0.290	9.009	15.914	12.918	49.734	38.250
13.	RAURD-185	2.629	0.720	13.722	18.116	10.250	41.946	38.530
14.	RAURD-190	1.815	0.571	10.115	14.399	9.089	40.091	38.493
15.	RAURD-193	2.643	0.736	12.181	17.178	10.248	46.270	38.687
16.	RAURD-195	2.424	0.746	12.058	16.636	9.667	45.711	36.750
17.	RAURD-200	2.314	0.690	10.202	15.262	8.735	44.100	37.577
18.	RAURD-214	3.094	0.595	12.703	16.813	10.424	44.206	38.283
19.	RAURD-220	2.068	0.692	12.702	16.655	10.213	43.357	38.520
20.	RAURD-221	2.332	0.599	12.403	16.276	10.265	36.191	38.357
21.	RAURD-241	2.174	0.931	12.499	15.811	10.056	45.118	38.770
22.	RAURD-242	2.521	0.936	11.329	18.175	9.419	46.139	37.333
23.	RAURD-245	2.503	1.070	11.220	16.590	10.419	46.642	37.767
24.	RAURD-246	2.586	0.736	14.365	17.279	9.528	43.248	37.433
25.	RAURD-273	2.283	0.912	11.547	16.647	10.814	43.175	37.263
26.	RAURD-07	3.058	0.854	14.227	16.925	9.845	42.264	38.557
27.	RAURD-23	2.709	0.574	12.510	16.104	8.327	36.549	38.700
28.	RAURD-25	2.413	0.761	13.188	16.383	10.382	43.801	38.763
29.	RAURD-32	2.659	0.832	11.068	16.714	9.702	42.334	38.357
30.	RAURD-63	2.151	0.723	12.043	17.119	9.523	44.749	38.730
31.	RAURD-69	2.527	0.142	11.410	15.847	8.335	47.651	38.643
32.	RAURD-78	2.622	0.914	12.077	16.869	10.224	46.460	39.310
33.	RAURD-205	3.412	0.793	13.777	15.506	10.587	43.602	37.530
34.	RAURD-212	2.553	0.571	11.724	16.286	9.383	42.205	37.663
35.	KRANTI	2.270	0.725	11.287	16.799	9.598	44.712	37.767
36.	PUSA BOLD	1.816	0.839	11.399	16.184	10.619	46.049	38.453
37.	VARDAN	2.195	0.545	12.805	17.815	11.058	44.720	37.640
38.	Rajendra Suflam	2.403	0.909	11.839	18.453	9.164	42.811	37.590
39.	Raj. Rai piccheti	2.448	0.731	12.742	15.941	10.402	44.045	38.603
40.	Rajendra Anukool	2.166	0.745	12.247	14.747	9.072	43.291	38.483
41.	RH- 30	2.432	0.863	13.510	16.594	9.631	44.150	38.553
42.	LAXMI	2.724	0.926	9.706	17.762	10.325	43.510	35.370
43.	JD-6	2.648	0.280	12.082	15.399	11.390	47.564	36.760
44.	EC-401574	2.090	0.283	12.076	17.110	7.875	47.831	39.410
45.	EC- 399788	2.667	0.669	12.816	15.905	9.834	38.190	38.073
46.	VARUNA (check)	2.893	0.915	10.945	15.391	9.381	54.850	37.693
	Mean	2.448	0.704	12.078	16.673	9.727	44.438	37.928
	Range Min	1.815	0.120	9.008	14.399	6.490	36.191	35.370
	Max.	3.412	1.070	14.364	20.142	12.918	54.849	39.410

Table 3: Distributing pattern of 46 genotypes of Indian mustard into seven clusters based on Euclidean analysis

Cluster group	No. of genotypes	Name of genotypes
Cluster I	2	RAURD-34 and LAXMI
Cluster II	1	RAURD-172
Cluster III	15	RAURD-153 RAJENDRA SUFLAM, RAURD-156, RAURD-154, RAURD-190, RAURD-200, VARUNA, RAURD-195, RAURD-164, RAURD-63, RAURD-212, KRANTI, RAURD-242, RAURD-32, RAJENDRA ANOOKUL
Cluster IV	16	RAURD-78, RAURD-273, PUSA BOLD, RAURD-193, RAURD-221, VARDAN, RH-30, RAURD-220, RAURD-214, RAURD 245, RAJ. RAI PICCHETI, RAURD-25, EC-399788, RAURD-166, JD-6 , RAURD-241
Cluster V	5	RAURD-07, RAURD-246, RAURD-205, RAURD-185, RAURD-168
Cluster VI	5	RAURD-89, RAURD-23, RAURD-69, RAURD-35, EC-401574
Cluster VII	2	RAURD-170, RAURD-171

Table 4: Average intra and inter cluster D^2 values among the cluster for 46 Indian mustard genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
Cluster I	1401.900	3835.051	3042.067	3666.052	7727.431	7903.497	13747.300
Cluster II		0.000	9199.968	7046.114	13117.73	16546.440	26446.780
Cluster III			997.473	1984.916	3866.201	2396.877	6183.694
Cluster IV				878.344	2238.122	3796.456	9616.077
Cluster V					1475.944	3707.577	8115.224
Cluster VI						1241.395	2725.211
Cluster VII							1301.560

Table 5: Mean values of clusters of different characters of 46 Indian mustard genotypes

Cluster	Fatty acid profile						
	Palmitic acid	Stearic acid	Oliec acid	Linoleic acid	Linolenic acid	Erucic acid	Oil content (%)
Cluster I	2.421	0.863	9.659	16.920	10.767	43.349	36.477
Cluster II	1.823	0.290	9.009	15.914	12.918	49.734	38.250
Cluster III	2.339	0.745	11.360	16.378	9.392	44.516	37.577
Cluster IV	2.421	0.748	12.371	16.453	10.445	44.201	38.254
Cluster V	2.945	0.804	14.061	17.594	9.941	41.572	37.581
Cluster VI	2.381	0.549	12.676	16.835	8.321	44.830	38.783
Cluster VII	2.760	0.229	12.627	18.074	6.851	50.387	37.975

Table 6: Contribution of different quality characters towards genetic divergence in Indian mustard

S. No.	Source	Contribution %
1	Palmitic acid	0.87
2	Stearic acid	0.48
3	Oliec acid	39.81
4	Linoleic acid	11.59
5	Linolenic acid	44.44
6	Erucic acid	0.77
7	Oil content (%)	2.03

distances (inter -cluster) was recorded between cluster II and VII followed by II and VI and cluster I and VII can be utilized by hybridization- selection breeding programme by involving genotypes in these clusters which can through useful transgressive segregants in the subsequent generations. However, it is also valuable considering genotypes within a cluster within a cluster with respect to a trait of interest as suggested by Teklewold *et al*. (2000), Chahal and Gosal (2002) and Keneni *et al*. (2005). Solitary genotypes (RAURD 172) in cluster II might have resulted from geographical barriers preventing gene flow or intensive selection for adaptive gene complexes as have been suggested by Teklewold *et al*. (2000). High cluster means, for palmitic acid, oleic acid and low erucic acid (cluster V). Cluster VII show low value of cluster mean for linolenic acid, high value for linoleic acid and erucic acid, whereas, high value of cluster mean for stearic acid in cluster I, and high cluster mean for linolenic acid and oil content showed in cluster II and VI respectively, reflected probability of getting better segregants and primary recombinants expected to more, in case if the genotypes of these clusters will be used in hybridization programme. Linolenic acid, oleic acid and linoleic acid pre-dominantly contributed maximum towards the genetic divergence (Table 6) along with less contribution of stearic acid and erucic acid. The suitable cluster were isolated cluster II and VII, cluster II and VI, cluster II and V and cluster II and I for future hybridization programme for the improvement of oil content and fatty acid profile. Clustering of genotypes into groups was mainly attributed by cumulative effects of individual traits. In general, this study indicates that there is a possibility of improving the fatty acid profile as well as the oil content of the genotypes through further breeding endeavour such as inter population crossing. The genotypes for hybridization may be chosen from widely separated clusters (fig. 1), as it is observed that there are several genotypes included in the crossing programme from widely separated clusters. The present investigation also revealed that diverse geographical origin of the genotypes could not necessarily be an index of variation and the factors other than geographical diversity such as genetic drift, selection pressure and environment may be responsible for discrepancy of genotypes.

ACKNOWLEDGEMENT

Authors are highly thankful to the Department of Soil Science TCA, Dholi, RAU-Bihar for providing laboratory facilities for the fatty acid analysis and also to DRMR, Sewar, Bharatpur, Rajasthan for Oil estimation of experimental material.

REFERENCES

Alemayehu, N. 1990. Yield and yield components of Ethopian mustard and rapeseed as affected by some agronomic practices. M. Sc. thesis, School of Graduate Studies of Alemaya Univ.

A. O. A. C. 1990. Official method of analysis. Association of Official Analytical Cereal Chemists.

Frankel, O. H., Brown, A. D. H. and Burdon, J. J. 1995. The conservation of plant biodiversity. Cambridge Uni. Press, Cambridge. UK. p. 299.

Genet, T., Labuschagne, M.T. and Hugo, A. 2004. Capillary gas chromatography analysis of Ethopian mustard to determine variability of fatty acid composition. *J. Sci. Food Agric.* **84:** 1663-1670.

Jatasra, D. S. and Parada, R. S. 1978. Genetic divergence in wheat under different environmental conditions. *Cereal Res. Comm*. **6:** 307-317.

Mahalanobis, P. C. 1936. On generalized distance in statistics. Proceedings of national institute of Science. **2:** 49-55.

Rao, C. F. 1952. Advanced statistical method in biometrical research. Ed. 1st Wiley and Sons Inc., New York.

Singh, P. 1983. Studies on genetic variability and diversity of rice. *Madras Agric. J.* **70(7):** 436-440.

Teklewold, A. 2005. Diversity study based on quality traits and RAPD markers and investigation of heterosis in Ethopian mustard. Ph. D. Thesis, George-August Univ. of Gottingen. Washington, DC, USA.

Wu, J.G., Shi, C. H. and Zhang, H. Z. 2006. Studying on developing calibration model of faty acid composition in intact rapeseed by near infrared reflectance spectroscopy. *Spectrose Spectr. Anal.* **26:** 259-262.

Zahan, M. I., Bhuiyan, M. S. R. and Hossain, M. S. 2008. Genetic divergence in Oleiferous *Brassica* species. *J. Sher-e-Bangla Agric. Univ.* **2(1):** 1-6.